

**IN THE UNITED STATES DISTRICT COURT
FOR THE DISTRICT OF DELAWARE**

GENENTECH, INC. and CITY OF
HOPE,

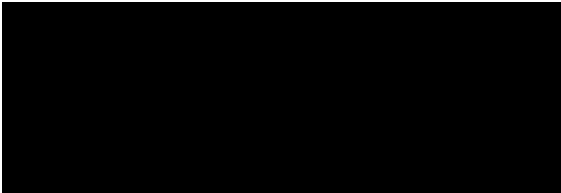
Plaintiffs,

v.

AMGEN INC.

Defendant.

)
)
)
) C.A. No. 17-1407-CFC-SRF
) (CONSOLIDATED)
)



) **PUBLIC VERSION FILED: May 7, 2020**
)

**REPLY BRIEF IN SUPPORT OF GENENTECH’S
MOTION TO DISMISS AMGEN’S FIFTEENTH COUNTERCLAIM
AND STRIKE ITS FIFTEENTH AFFIRMATIVE DEFENSE**

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The Court's prior rejection of Amgen's request to add its inequitable conduct claim, D.I. 628, remains the right result. The Court's subsequent ruling granting Genentech leave to amend, D.I. 629, does not change the analysis under Rule 15, and even if it did, Amgen's theory of inequitable conduct is legally unsupported. The Federal Circuit has made it clear that inventors cannot be accused of inequitable conduct for "hiding" information that indisputably was disclosed to the Patent Office during prosecution.

ARGUMENT

I. RULE 15 DOES NOT AUTHORIZE THE INEQUITABLE CONDUCT THEORY THE COURT HAS ALREADY DEEMED UNTIMELY.

Rule 15 does not resuscitate the inequitable conduct claim the Court has already excluded on the basis that it could and should have been brought months ago.

1. The prevailing interpretation of Rule 15(a) allows a party to add new material in response to amended pleadings only where "the breadth of the changes in the amended response . . . reflect the breadth of the changes in the amended complaint." *Teva Pharm. USA, Inc. v. Forest Labs., Inc.*, 2016 WL 7325511, at *1 n.1 (D. Del. June 16, 2016) (quoting *Elite Entm't, Inc. v. Khela Bros. Entm't*, 227 F.R.D. 444, 446 (E.D. Va. 2005)); *Sirona Dental Sys., Inc. v. Dental Imaging Techs. Corp.*, 2012 WL 3929949, at *3 (D. Del. Sept. 10, 2012). This so-called "moderate approach" has been endorsed by the Federal Circuit, other district courts, and

commentators because it permits courts “to effectively manage the litigation” and ensures that amendments do not give defendants the ability to “revive[] without cause . . . claims that would otherwise be barred or precluded.” *E.E.O.C. v. Morgan Stanley & Co., Inc.*, 211 F.R.D. 225, 227 (S.D.N.Y. 2002); *see also Unigene Labs., Inc. v. Apotex, Inc.*, 655 F.3d 1352, 1359-60 (Fed. Cir. 2011) (applying Second Circuit law); *Patel v. Pandya*, 2016 WL 3129615, at *2 (D.N.J. June 2, 2016) (explaining “the moderate approach”).

The same reasoning supports dismissal of Amgen’s counterclaim and affirmative defense in this case. Amgen makes no effort to substantiate its assertion that Genentech’s amended complaint “substantively expanded the scope of the case,” D.I. 665 at 9, and for good reason—there is no connection at all between the modest changes in Genentech’s amendment and the new claims Amgen seeks to add. Most of Genentech’s amendments do not relate to the Kao patent at all. The one that does—a new claim of infringement under 35 U.S.C. § 271(g)—does not change in any material way the theory of the case Genentech has been pursuing for more than two years; it merely alleges Amgen’s infringement is actionable under an additional statutory subsection. The only expansion of the case would come from Amgen’s amendment. Its inequitable conduct allegations have not yet been the subject of discovery, *see infra* pp. 5-6, and would require the Court to conduct a separate bench trial after the issues of infringement and validity

are resolved by the jury.

2. Amgen's assertion that "longstanding precedent in this Court" forecloses Genentech's motion, D.I. 665 at 4, is simply incorrect. We remain mindful that the Court applied a more permissive standard in the related Herceptin litigation, but before that the most recent decisions in this District adopted the rule advocated by Genentech. *See Teva*, 2016 WL 7325511; *Sirona*, 2012 WL 3929949. Amgen argues that those cases involved "purely ministerial amendments," D.I. 665 at 8, but whatever their nature the rule *Sirona* and *Teva* adopted was clear, and fully consistent with how courts and commentators alike have addressed the question: amendments that do not change the breadth of the complaint do not "throw the door open to entirely new claims and defenses." *Sirona*, 2012 WL 3929949, at *3; *Teva*, 2016 WL 7325511 at *1.

Nor has Amgen cited any decision applying the minority approach to permit the addition of a defense previously denied as untimely and unrelated to the amendment. This case is a poor candidate for breaking new ground. Genentech's motion for leave, attaching its proposed amended complaint, was on file by February 2019, months before fact discovery closed in July 2019. Instead of consenting to this amendment and proposing its own—something the Court has noted Amgen was in a position to do, D.I. 628 at 3—Amgen cluttered the docket and wasted the Court's resources with a meritless opposition arguing that (i)

Genentech’s amendment predating the Scheduling Order’s deadline for amendments was nevertheless “untimely,” and (ii) the Court should ignore controlling authority interpreting § 271(g) and instead adopt the views expressed in a dissenting opinion. *See* D.I. 293; D.I. 629.

Amgen’s February 2020 amendment is even less timely, more prejudicial, and more disruptive to this case than Amgen’s identical September 2019 amendment that the Court properly rejected on the basis that Amgen waited too long. The notion that an unrelated amendment, of which Amgen was aware during the time it delayed unduly, somehow can excuse Amgen’s behavior lacks any basis in either precedent or fairness. It would reward Amgen for filing a meritless opposition that delayed Genentech’s amendment by nearly a year. Not even under the more permissive standard and associated cases Amgen cites has such a result been countenanced.¹

3. Amgen insists that the Court’s prior finding of undue delay has been rendered “irrelevant.” D.I. 665 at 11. But all the reasons justifying the earlier

¹ Amgen accuses Genentech of misrepresenting the state of the law in this District by “contend[ing] that none of the decisions cited by the Court [in the Herceptin ruling] involved counterclaims first asserted after the deadline to amend.” Opp. 6. Amgen should read Genentech’s brief more closely. It noted—correctly—that none of those cases “appear[ed] to address the impact of a missed deadline in a scheduling order” nor the impact of “the amended response includ[ing] defenses that the Court has already held could have been pursued prior to that deadline.” Opening Br. 3 n.1. As stated, those are relevant considerations that do exist in this case.

ruling still exist. Genentech is still left with far less time to take discovery to defend itself than it would otherwise have had. Nor is it true, as Amgen suggests, D.I. 665 at 7-8, that the relevant discovery lies uniquely in Genentech's control. Were the inequitable conduct claim allowed, Genentech would justifiably seek discovery from a number sources outside its control, including former Genentech employees associated with the Kao patent's prosecution and third parties who peer-reviewed or praised the Kao inventors' work. Discovery would also include Amgen witnesses. For example, Amgen claims that the data in the Trexler-Schmidt 2010 publication was material because, "if disclosed [sic – it *was* disclosed, *see infra* pp. 6-7]," the publication "would have shown that [the Kao inventors] did not discover a method to prevent disulfide bond reduction as claimed in the patent." D.I. 665 at 12. This would be news to the Amgen scientists who acknowledged years ago, before this litigation, that Trexler-Schmidt 2010 is fully consistent with the Kao inventors' claim to have invented a method of preventing reduction.² Pursuing the discovery necessary to defend against this allegation of fraud, long after the fact-discovery deadline, would be burdensome

² *See* Ex. 1 (Mvasi aBLA, AMG00005501) at 63 ("Studies reported in the literature indicate that reduction of IgG1 antibodies may potentially occur during cell harvest operations (Trexler-Schmidt et al, 2010)."); Ex. 2 (Hutterer et al. 2013) at 608 ("Reduction has been shown to be virtually eliminated by maintaining dissolved oxygen (DO) levels during harvest operations", *citing* Trexler-Schmidt 2010); *see also* Ex. 3 (Nehring Tr.) at 103 (noting "that much of the industry is very aware of" Trexler-Schmidt 2010).

even in ordinary times. In the present situation, the burden on Genentech will be even greater and far more prejudicial.

II. AMGEN FAILS TO PLEAD A VIABLE THEORY OF INEQUITABLE CONDUCT.

The Court can grant Genentech's motion without resolving the parties' dispute over Rule 15 because the theory of inequitable conduct Amgen advances is meritless even had it been timely pursued.

1. Amgen's theory falters on one inescapable, undisputed fact: the examiner had all the data that Amgen alleges "was deliberately withheld from, and misrepresented to, the examiner." D.I. 665 at 15. The examiner received the data in question when the same inventors Amgen accuses of deception supplied him during prosecution with the Trexler-Schmidt 2010 publication that features the data Amgen alleges was withheld. As Genentech explained in its opening brief, this is fatal to Amgen's claim because, where the Patent Office actually received the information the infringer alleges was material to patentability, an inequitable conduct allegation cannot succeed as a matter of law. *See* D.I. 658 at 6-7.

Genentech's disclosure of Trexler-Schmidt 2010 sets this case apart from those Amgen cites. In *Delano Farms Co. v. California Table Grape Comm'n*, 655 F.3d 1337, 1350 (Fed. Cir. 2011) and *Cargill, Inc. v. Canbra Foods, Ltd.*, 476 F.3d 1359, 1364 (Fed. Cir. 2007), the patentee was alleged to have withheld material information from the Patent Office. Here the inventors concededly supplied it.

Nor does this case resemble *Wyeth Holdings Corp. v. Sandoz, Inc.*, 2012 WL 600715 (D. Del. Feb. 3, 2012), where the defendant had alleged that “the Examiner did not have [the material] information and had no way of independently learning it.” *Id.* at *11; *see also id.* at *12 (“Sandoz appears to assert that this data was never provided by Wyeth to the Examiner in any form”).

Bristol-Meyers Squibb Co. v. Rhone-Poulenc Rorer, Inc., 2002 WL 59429 (S.D.N.Y. Jan. 16, 2002), is even further afield. In that case, a patent agent deliberately withheld a known material reference from both the prosecuting attorney and the Patent Office. *Id.* at *17. Both the district court and the Federal Circuit rejected the argument that the patentee could cure its agent’s inequitable conduct by subsequently disclosing the reference during prosecution of *a different application*, after the examiner had already decided to issue the other patent. *Id.* at *18; *see also Bristol-Myers Squibb Co. v. Rhone-Poulenc Rorer, Inc.*, 326 F.3d 1226, 1241 (Fed. Cir. 2003) (explaining that “the issue is [the applicant’s] intent during the prosecution of the original application,” and therefore “disclosure during reissue is irrelevant to the inquiry of whether [applicant] acquired the [original] patent by engaging in inequitable conduct”). That holding has no bearing here, where Genentech supplied the supposedly contrary data in the *same* prosecution and before the examiner’s decision to grant the patent.

2. Confronted with this inconvenient fact Amgen instead quibbles about

when the disclosure occurred. D.I. 665 at 15 (complaining that Trexler-Schmidt 2010 was submitted after filing of the priority patent application). But even in Amgen's telling of it, there was nothing nefarious about the timing of Genentech's submission. And regardless, no case holds that disclosing information to the Patent Office late in prosecution but before the patent is allowed is a basis for inequitable conduct. On the contrary, the case law adopts a bright-line rule that is dispositive of Amgen's defense: if information is disclosed it cannot be considered withheld. *Rothman v. Target Corp.*, 556 F.3d 1310, 1329 (Fed. Cir. 2009). And when information is provided to the examiner, the examiner has "discretion to reject or accept an applicant's arguments based on the examiner's own conclusions regarding the prosecution record." *Id.* In those circumstances there can be no inequitable conduct.

Finally, citing *Rohm & Haas Co. v. Crystal Chem. Co.*, 722 F.2d 1556 (Fed. Cir. 1983), Amgen argues that the submission of Trexler-Schmidt 2010 does not excuse Genentech from correcting its supposed misstatements to the Patent Office. *See* D.I. 665 at 15-16. But again, Amgen misreads the case law. The applicant in *Rohm & Hass* made intentional, admitted misrepresentations of material facts. The "narrow issue" the Federal Circuit addressed was whether an applicant's "voluntary efforts during prosecution . . . *knowing that misrepresentations have been made to the examiner of his application*, can ever alleviate its effect." *Id.*

at 1571-72 (emphasis added). Here, Amgen cannot point to any misrepresentations Genentech supposedly needed to cure; rather, Amgen's allegation is that Genentech withheld data that it indisputably provided to the Examiner during prosecution. *See* D.I. 658 at 9-10. This case has nothing to do with cure, as the alleged "withholding" did not occur as a matter of law. *Rothman v. Target Corp.*, 556 F.3d 1310, 1329 (Fed. Cir. 2009); *Young v. Lumenis, Inc.*, 492 F.3d 1336, 1349 (Fed. Cir. 2007); *Akzo N.V. v. ITC*, 808 F.2d 1471, 1482 (Fed. Cir. 1986).

CONCLUSION

For the foregoing reasons, Genentech respectfully requests that the Court strike the portion of Amgen's Fifteenth Affirmative Defense directed to the Kao Patent under Rule 12(f) and strike, or dismiss under Rule 12(b)(6), Count 15 of Amgen's counterclaims.

Respectfully Submitted,

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Exhibit 1

**THIS DOCUMENT HAS
BEEN REDACTED IN ITS
ENTIRETY**

Exhibit 2

Monoclonal antibody disulfide reduction during manufacturing

Untangling process effects from product effects

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Keywords: antibody disulfide reduction, free cysteine, harvest, capillary electrophoresis, CE-SDS

Abbreviations: CCF, cell culture fluid; CHO, Chinese hamster ovary; DO, dissolved oxygen; DTT, dithiothreitol; DTNB, 5,5'-dithiobis-(2-nitrobenzoic acid) (Ellman's reagent); EOP, end of production; HCCF, harvested cell culture fluid; IAM, iodoacetamide; IgG, immunoglobulin G; mAb(s), monoclonal antibody(ies); MEA, micro-extractor automated instrument; NADPH, nicotinamide adenine dinucleotide phosphate; NEM, N-ethylmaleimide; NR CE-SDS, non-reduced capillary electrophoresis with sodium dodecyl sulfate; PAT, process analytical technology; PPP, pentose phosphate pathway; SDS, sodium dodecyl sulfate; t0, initial time point

Manufacturing-induced disulfide reduction has recently been reported for monoclonal human immunoglobulin gamma (IgG) antibodies, a widely used modality in the biopharmaceutical industry. This effect has been tied to components of the intracellular thioredoxin reduction system that are released upon cell breakage. Here, we describe the effect of process parameters and intrinsic molecule properties on the extent of reduction. Material taken from cell cultures at the end of production displayed large variations in the extent of antibody reduction between different products, including no reduction, when subjected to the same reduction-promoting harvest conditions. Additionally, in a reconstituted model in which process variables could be isolated from product properties, we found that antibody reduction was dependent on the cell line (clone) and cell culture process. A bench-scale model using a thioredoxin/thioredoxin reductase regeneration system revealed that reduction susceptibility depended on not only antibody class but also light chain type; the model further demonstrates that the trend in reducibility was identical to DTT reduction sensitivity following the order IgG1 λ > IgG1 κ > IgG2 λ > IgG2 κ . Thus, both product attributes and process parameters contribute to the extent of antibody reduction during production.

Introduction

The target specificity, favorable pharmacokinetics and pharmacodynamics, and stability of monoclonal human immunoglobulin gamma (IgG) antibodies have resulted in their widespread use in the biopharmaceutical industry.^{1,2} Commercial therapeutic antibody production is a complex but fairly well established process, typically involving expression in Chinese hamster ovary cells (CHO), harvesting of the secreted protein, and a series of chromatography steps to remove impurities. Reduction of antibody interchain disulfide bonds during manufacturing operations has recently been the subject of much interest.^{3–5} This phenomenon is observed when extending the time that the antibody remains in the cell culture fluid (CCF) or harvested cell culture fluid (HCCF) in the “harvest” step of production. This harvest step includes separation of cells from the media prior to the first column purification.

Process-induced antibody disulfide bond reduction has been observed inconsistently at large scale processes and is not typically observed with standard bench-scale (up to 10 L) models.⁵ This reduction has been attributed to certain enzymes that are released from the intracellular compartments of lysed cells. Components in the thioredoxin reduction pathway, including thioredoxin reductase and NADPH, have been proposed as the principal underlying contributor for this antibody disulfide bond reduction.^{3,4} Reduction has been shown to be virtually eliminated by maintaining dissolved oxygen (DO) levels during harvest operations.⁵ In addition, the cysteine/cystine redox couple, which is present in the growth media, may affect disulfide bond formation, reduction, and rearrangement.⁶ Likewise, many other media components, such as certain metal ions and their complexes, are likely to affect the reduction potential during the harvest procedure.^{5,6}

In these studies, cell lysis and an anaerobic environment both promoted antibody reduction during harvest;^{5,6} therefore,

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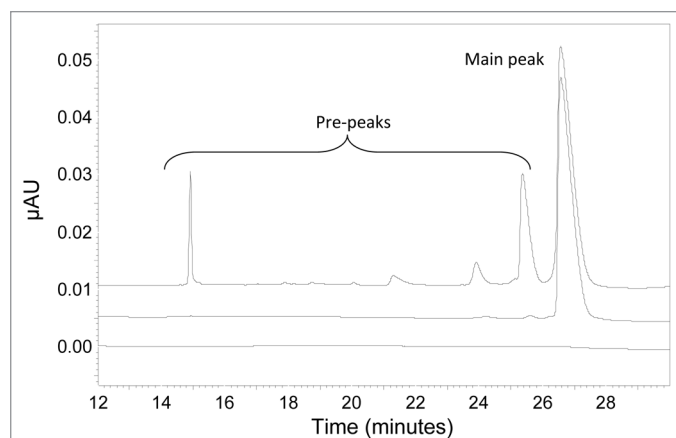


Figure 1. NR CE-SDS Electropherograms. Partially Reduced mAb (top), Purified mAb (middle), and Blank (bottom).

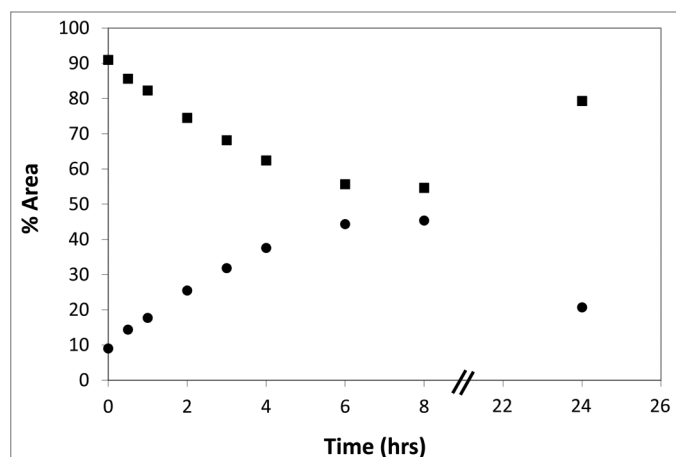


Figure 2. Reduction of mAb A in Small Scale Reduction Model as a Function of Time. Pre-peaks (circles) and Main peak (squares).

it is clear that adequate process understanding and control is necessary to minimize or eliminate disulfide bond reduction induced by manufacturing procedures. In addition to variation due to manufacturing processes, differences between products were observed.⁵ Because cell cultures, cell lines, and the products themselves can vary in cell cultures expressing two different antibody products, the underlying causes for these reduction differences could not be determined. The study presented here explores the relationship between reduction and process variables, separating the influence of process and products to demonstrate that CHO cell line or cell culture process can dramatically influence reduction during harvest operations and that the antibody class and light chain type also influences the extent of that reduction.

Results

Small scale model. Harvest-related disulfide reduction has been reported as highly dependent on process scale and has been

reported in some scaled-up, but not bench-scale, processes.⁵ This effect of scale may be attributed to the maintenance of oxygen in small-scale harvests, which may preserve disulfide bonds. Typically, bench-scale experiments are open to the air, which allows more efficient oxygen transfer than typical manufacturing-scale (15,000 to 20,000 L) cell culture production. Bench-scale experiments also use different centrifuge equipment, introducing the possibility of different degrees of cell shearing during removal of debris. To facilitate harvest reduction experiments, a small-scale model, similar to previously described models,⁵ was developed. A “worst case” reduction model of cell culture extract was generated by mechanically shearing 2 L of whole cell culture fluid (CCF) used for production of an IgG1κ mAb (mAb A), transferring the sheared CCF into a 3 L bioreactor, and sparging the resultant slurry with nitrogen to simulate the anaerobic environment of the commercial scales. Samples were taken at 0, 0.5, 1, 2, 4, 8, and 24 h and immediately frozen at -70°C. Non-reduced capillary electrophoresis with sodium dodecyl sulfate (NR CE-SDS) was performed on all samples to measure the degree of interchain disulfide bond breakage. Representative electropherograms of a partially reduced antibody, a properly disulfide-linked antibody, and a blank are shown in Figure 1. This figure shows that the peaks in the pre-peak region of the electropherogram increase in intensity relative to the main, properly disulfide-linked, peak. These pre-peaks have been shown to be light chain (L), heavy chain (H), and combinations of the two chains (HL, HH, HHL).⁷ Because size exclusion chromatography indicates that reduction does not result in disassembly of the antibody chains, the NR CE-SDS pre-peaks represent properly assembled antibodies with one or more broken interchain disulfide bonds. The relative area associated with the pre-peaks and main peak were used to monitor interchain disulfide reduction in a series of harvest experiments. Results showing antibody reduction in mAb A for up to 24 h after cell shearing in the small-scale model are shown in Figure 2. An increase in the percentage of pre-peaks over time is observed, from 9% at the initial time point to ~45% at 8 h. This increase in the percentage of pre-peaks replicates previously published results⁵ and demonstrates that the small-scale model is capable of inducing and monitoring disulfide bond reduction. It is worth noting that the pre-peak level decreases after 8 h, and it is only ~12% by 24 h, indicating that disulfide bonds can reform. This observation is consistent with previously published results.⁵

Product, cell line and process. Partial disulfide bond reduction behavior was probed with multiple Amgen therapeutic antibodies and cell lines. Three products, an IgG2λ (mAb B), an IgG2κ (mAb C), and mAb A, the IgG1κ discussed above, were tested by shearing end of production cells and subjecting the lysed CCF to nitrogen sparging in the small-scale model at 25°C. Figure 3 displays the relative amount of intact antibody for each of these products as a function of time. Although the IgG1κ results demonstrate that an interchain disulfide can be reduced in this antibody type using this small-scale model, no changes were seen in mAb C (IgG2κ) or mAb B (IgG2λ). This lack of reduction under these reduction promoting conditions has not been previously reported, and indicates tight controls of air sparging

or cell shearing are not necessary for all antibody production processes. This comparison of end of production CCF shows stark differences in behavior, but does not distinguish between the effects of product, cell line, or cell culture process. While the mAb C (IgG2 κ) titer and cell density are fairly low, and that might account for the difference, both the mAb A (IgG1 κ) and the mAb B (IgG2 λ) have relatively high titers and cell densities, as shown in Table 1. IgG2s, such as the mAb B, are known to be less susceptible to reduction by thioredoxin;⁸ however, there could also be differences between the cell lines or processes that could contribute to these observations.

Direct comparison between cell lines is complicated due to the differences that may arise through the transfection process. Both copy number and insertion site can vary from clone to clone, and both of these parameters may also affect cell growth, viability, productivity, and metabolism.^{12,13} Therefore, to partially disentangle the effect of product, cell line and process on the level of reduction, end of production cells from these three products were lysed, and the original product was removed via Protein A affinity to create soluble cellular component material. The reduction activity was shown to be maintained through this type of processing by Trexler-Schmidt et al.⁵ The results in Table 2 illustrate the difference in NR CE-SDS % Main peak between the t0 and 8 h samples for several combinations of cells and purified products. To determine whether this material remained active, purified mAb A was spiked back into its own soluble cellular components and held under nitrogen overlay for 8 h. When mAb A sheared cell broth was reconstituted in this manner, the difference in NR CE-SDS % Main peak was ~45%, identical to the small-scale model results, which indicates that the reducing activity was preserved through this processing step. This is consistent with the experiment performed by Trexler-Schmidt et al.⁵ When purified mAb D (IgG2 κ) was spiked into mAb A soluble cellular components, little reduction (0.9% reduction in % main peak) was observed, showing that mAb D reduction is more resistant to these conditions. Thus, the product will influence the degree of reduction observed in the harvest process. To test the effects of cell line and cell culture process independent of product, purified mAb A was spiked into mAb B and mAb C soluble cellular components. Although mAb A is susceptible to reduction in its own soluble cellular components, little reduction was observed when it was incubated in either mAb C or mAb B components (1.5% and 2.7% reduction in % main peak, respectively). All of the materials were carefully sparged with nitrogen during processing, and the soluble cellular components were prepared and used within 30 min of cell lysis. In addition, repeat analysis of the (mAb B) IgG2 λ spike into the mAb A (IgG1 κ) yielded identical results, as did intermediate time points for the other conditions. The lack of reduction for these conditions must be due to differences in the reducing power of cellular component samples because the product is identical. Disulfide reducing ability of the cellular component sample could arise from differences in the cell line, such as differences in expression of thioredoxin or thioredoxin reductase, or differences in availability of NADPH due to regulation of the pentose phosphate pathway (PPP). Differences in reducing power of the soluble cellular component sample could also arise from

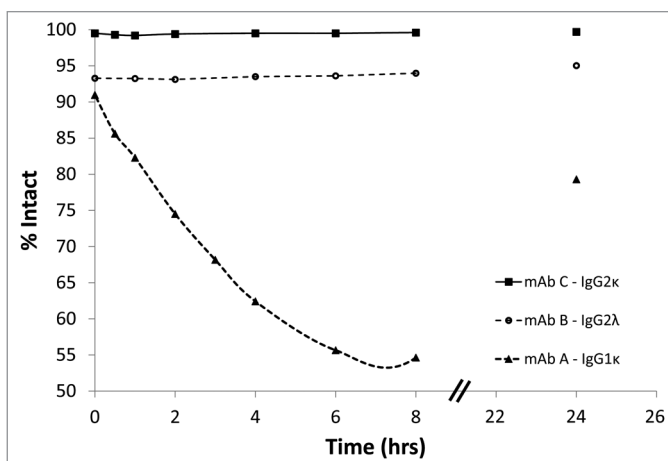


Figure 3. Reduction Behavior of Different Products and Cell Lines over time. mAb A (triangles), mAb B (circles), and mAb C (squares).

the cell culture process differences, either indirectly, by influencing expression of thioredoxin, expression of thioredoxin reductase, and utilization of the PPP, or directly, by differing levels of redox active media components such as cystine/cysteine and copper. In the case of mAb C, the measured thioredoxin reductase activity is lower than that for mAb A (Table 1); however, this could be due to the lower cell density. In contrast, the cell density is similar for mAb B, and therefore the cell line and process can be determined to have a significant effect on reducing power. The presence of substantial thioredoxin reductase activity in these cell lysates is not unexpected because some apoptosis, which will release intracellular contents, inevitably occurs during cell culture. This means, however, that the difference in reducing power cannot be attributed to thioredoxin system activity alone because the thioredoxin reductase activity was higher in the mAb B lysate than the mAb A lysate. Therefore, either the other redox active components of the system have a major affect or substantial differences in the availability of NADPH exist. Taken together, the results suggest that the cell line (clone) or cell culture process play a key role in harvest-related reduction.

Product properties. As described above, the reconstituted extract model, demonstrates that striking differences exist in susceptibility to reduction among antibody products. Previously published studies have shown that antibody sub-classes differ in sensitivity to disulfide bond reduction.⁸ Differences in reduction susceptibility due to light chain type have not previously been observed for thioredoxin catalyzed reduction, but have been shown using chemical reductants.⁹⁻¹¹ A chemical model system was developed to investigate antibody type (IgG1 and IgG2) and light chain type sensitivity to thioredoxin catalyzed reduction. As illustrated in Figure 4, reduction sensitivity is dependent on both antibody class and light chain type. Reduction sensitivity, in decreasing order, is IgG1 λ , IgG1 κ , IgG2 λ , IgG2 κ . This trend held true for all of the additional molecules we have tested, and for different stoichiometric ratios of the reagents and antibody (data not shown). Sensitivity to antibody subclass has also been reported for other reductants, such as DTT.^{9,10} The reduction sensitivity trend

Table 1. End of production cell densities, viability and titer for mAbs A, B and C

Product	Viable Cell Density (cells/mL)	Viability (%)	Titer (g/mL)	Thioredoxin Reductase Activity (μmol/min/mL)
IgG2κ (mAb C)	7.0x10 ⁶	75.4	1.3	Below detection limit
IgG2λ (mAb B)	17.2x10 ⁶	62.4	4.7	0.16
IgG1κ (mAb A)	27.0x10 ⁶	78.9	4.4	0.04

Table 2. Influence of product and cell line/process on reduction

Soluble Cellular Component	Purified mAb	Difference in NR CE-SDS % Main peak
IgG1κ (mAb A)	IgG1κ (mAb A)	46.3%
IgG1κ (mAb A)	IgG2κ (mAb D)	0.9%
IgG2κ (mAb C)	IgG1κ (mAb A)	1.5%
IgG2λ (mAb B)	IgG1κ (mAb A)	2.7%

Difference in NR CE-SDS % Main peak, 8 h, relative to initial.

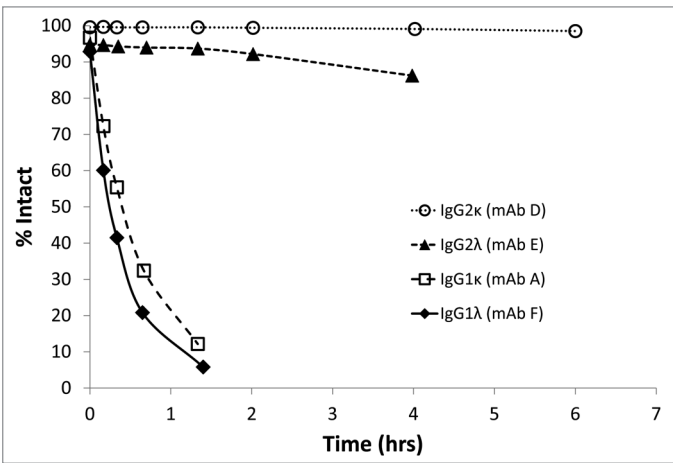


Figure 4. Influence of Product on Reduction using Thioredoxin System. Intact antibody, as measured by % Main peak in the NR CE-SDS analysis as a function of time.

was similar between the thioredoxin system and with DTT (Fig. 4 and 5). This comparison indicates that differences in reduction during harvest are the result of overall reducing potential of the system and the antibody type, and not any specific interactions between thioredoxin and certain antibody types.

Equipped with the knowledge of the reducibility trend IgG1λ > IgG1κ > IgG2λ > IgG2κ, a general understanding of the product contribution to the risk of process-induced reduction can be made prior to expression of products. This general understanding can be further refined by putting a small amount of purified product into either the chemically-defined thioredoxin reducing system, or by making kinetic measurements of the reducibility of the product by DTT.

Discussion

Process-induced partial antibody disulfide reduction is an active topic of discussion in the literature and with regulatory

authorities. This phenomenon is caused by shearing of cells, resulting in the release of intracellular components, and requires an anaerobic environment. Different cell lines and processes have been demonstrated to have strikingly different reduction responses, e.g., mAb B soluble cellular components having less than 1/10th the reducing power of those of mAb A. The difference in reducing power cannot be attributed to differences in thioredoxin and thioredoxin reductase levels, as measurement of thioredoxin reductase shows that it is higher in some of the cell lysates that show no reduction. Therefore, these differences must stem from other redox active components in the media, or more likely, from differences in NADPH availability and regulation of the PPP.

The susceptibility of products to reduction by thioredoxin has been demonstrated to be dependent on antibody class and light chain type, IgG1λ > IgG1κ > IgG2λ > IgG2κ, with potentially some sequence dependency within each range. The susceptibility of the antibody classes to thioredoxin catalyzed reduction follows the same trend as antibody disulfide reduction by DTT. Therefore, a general understanding of product reducibility is available prior to expression of the product, and a more refined understanding of its susceptibility to reduction is possible with only micrograms of material in a chemically-defined system.

With the understanding of the reducing power of the cell line and process, screening of cell lines and cell culture conditions is possible. Combining process knowledge with the antibody class, a good understanding of the overall reduction behavior can be obtained early in process development.

Materials and Methods

Materials. Cell culture fluid and purified antibodies were produced at Amgen using standard manufacturing procedures. Reagents were obtained from Sigma-Aldrich unless otherwise specified.

Cell shearing. Complete cell lysis of end of production (EOP) cell culture fluid, which contains both cells and the media containing product, was achieved by high-pressure homogenization using a Microfluidics M-110Y high shear fluid processor. Homogenization was performed with a single pass at 8,000–10,000 psi. Complete lysis was verified using the Roche Innovatis Cedex AS20 cell counter.

Small-scale reduction model. A 3 L glass stirred-tank bioreactor (Applikon Corporation) controlled by a customized DeltaV distributed control system (DCS) was used to evaluate harvest conditions. Processed cells were transferred to this bioreactor. Agitation was set at 250 rpm. Temperature was controlled to 8–10°C by passing chilled water through a thermal well in the bioreactor. Room temperature conditions were

unregulated, at -22°C . Dissolved oxygen (DO) was measured using a Mettler Toledo DO probe connected to a Rosemont Transmitter. DO was lowered by sparging nitrogen gas through a drilled tube sparger with a flow sufficient to achieve a zero response for dissolved oxygen. To achieve oxygen at the 100% level, air was passed through the drilled tube sparger at 100 to 200 mL/min. DO, temperature, airflow and agitation data were collected by the DeltaV DCS and archived into a PI data historian (OSIsoft).

Reconstituted extract model. Cell culture fluid (CCF) depleted in antibody product was generated using a batch binding process to remove existing monoclonal antibodies (mAbs). CCF was transferred to a 250 mL polycarbonate bottle (Nalgene) and homogenized using a Tissue Tearor™ (Biospec Products) for one minute of homogenization to ensure complete cell breakage. During homogenization, a nitrogen (N_2) gas overlay was applied. MabSelect SuRe™ Protein A affinity resin (GE Healthcare) was washed twice with an equilibration buffer of 100 mM NaCl, 25 mM Tris, pH 7.4, dried by vacuum over a nylon membrane, and applied in excess directly to the bottle. The mixture was placed on a rocker for 10 min to facilitate binding. The solution was centrifuged for 5 min x 1000 rpm in 50 mL conical tubes to pellet the resin. The supernatant was extracted from each tube and sparged with N_2 to form the soluble cellular components, and used within 30 min of production.

Sample antibody drug substance was added to a separate 15 mL polypropylene centrifuge tube and brought to a total volume of 7 mL with the soluble cellular component material to give a final antibody concentration of 3 mg/mL. An N_2 overlay was applied to each tube. The tubes were covered with laboratory paraffin film and placed in a digitally controlled water bath set at 10°C . One mL aliquots were pulled at 0, 4 and 8 h and immediately frozen at -80°C prior to analysis by NR CE-SDS.

Reduction by thioredoxin system. The roles of thioredoxin and thioredoxin reductase (TR) have previously been described as nicotinamide adenine dinucleotide phosphate (NADPH)-dependent cellular protein disulfide reductases.^{14,15} An in vitro lab-scale model using this complex was optimized using recombinant human thioredoxin (Sigma), NADPH (Calbiochem), in excess and thioredoxin reductase from rat liver (Sigma). A polypropylene 2 mL cryogenic vial (Corning) was sparged for 1 min with N_2 prior to being sealed in a borosilicate septa vial (I-Chem). In a separate 1.5 mL microcentrifuge tube, 825 μL phosphate buffered saline, 14 μL NADPH (10 mM), 10 μL human thioredoxin solution (0.5 mg/mL) and antibody drug substance were combined to give a final volume of 1 mL and antibody concentration of 4 mg/mL. The reaction was initiated with the addition of 18 μL TR solution (7 μM) and transferred immediately into the sealed vial using a syringe. The septa vials were placed within a temperature-controlled water bath at 10°C with an applied overlay of N_2 to exclude oxygen. Aliquots of 100 μL were taken at each time point, quenched immediately with 8.7 μL N-ethylmaleimide (NEM) at 250 mM and frozen at -80°C prior to analysis by NR CE-SDS.

Reduction by DTT. Partially reduced mAbs, with majority of the interchain disulfide bonds broken, were generated

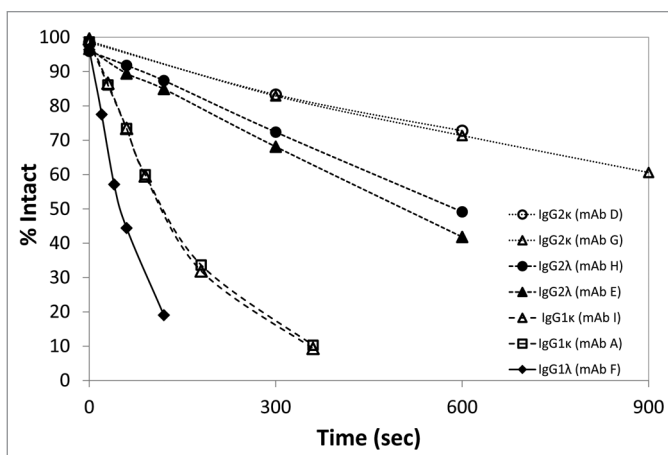


Figure 5. Influence of Product on Reduction using DTT. Intact antibody, as measured by % Main peak in the NR CE-SDS analysis as a function of time.

by incubating mAbs at the concentration of 2 mg/mL in 50 mM TRIS-HCl, pH 7.5 (Teknova) with 2 mM DTT (Geno Technology) at ambient temperature. Aliquots were taken at multiple time points and the reduction was quenched by immediately adding NEM (MP biomedical) to a final concentration of 25 mM. A non-reducing Caliper CE-SDS assay was performed to measure the level of reduction.

Thioredoxin reductase activity. Thioredoxin reductase activity of lysates was assessed using a colorimetric 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB) kit (Cayman Chemical). Briefly, lysed CCF was added to a pH 7 sample buffer containing 50 mM potassium phosphate, 50 mM potassium chloride, 1 mM EDTA, and 0.2 mg/mL bovine serum albumin, at a final dilution factor of 1:10. For each sample a matrix control was made by adding 20 μM sodium aurothiomalate (final concentration). Excess NADPH and 0.5 mM DTNB (final concentration) were added to each sample, matrix control, blank, and positive control (rat liver thioredoxin reductase). Light absorbance was monitored at 405 nm for 5 min, and the activity of thioredoxin reductase in $\mu\text{mol}/\text{min}/\text{mL}$ was calculated by taking the difference in slopes between the sample and the matrix control, dividing by the extinction coefficient and path length, and multiplying by the dilution factor.

Non-reduced CE-SDS. Harvested cell culture fluid samples were prepared using an automated robotic platform, as previously described.¹⁶ Briefly, samples were centrifuged at 13,000 rpm for 1 min and loaded onto a Micro-Extractor Automated Instrument (MEA, PhyNexus). PhyTip® 200 μL Columns with 20 μL protein A affinity resin protein A tips were used to remove host cell proteins. Non-reducing sample buffer with a final concentration of 7 mM NEM, 57 mM sodium phosphate, 1.9% SDS, pH 6.5 was added to the purified samples. Incubation was set for 5 min at 60°C and samples were injected onto a 30 cm bare fused silica capillary with a 20 cm effective length and 50 μm inner diameter using electrokinetic injection. Separation was performed using CE-SDS gel (Beckman Coulter) and 15 kV effective voltage, and detection was by UV light absorbance at 220 nm.

Non-reduced Caliper CE-SDS. A LabChip 90 (Caliper Life Sciences) was used to separate SDS bound proteins through a sieving polymer based on the hydrodynamic size of the SDS-protein complex.¹⁷ HT Protein Express Sample Buffer (Caliper Life Sciences) was combined with iodoacetamide (IAM) to a final IAM concentration of approximately 5 mM. A total of 5 μ L antibody sample at approximately 1 mg/mL was mixed with 100 μ L of the IAM containing sample buffer. The samples were incubated at 75°C for 10 min. The denatured proteins were analyzed by LabChip 90 with the “HT Protein Express 200” program.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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Exhibit 3

**THIS DOCUMENT HAS
BEEN REDACTED IN ITS
ENTIRETY**

**IN THE UNITED STATES DISTRICT COURT
FOR THE DISTRICT OF DELAWARE**

GENENTECH, INC. and CITY OF HOPE,)	
)	
<i>Plaintiffs,</i>)	C.A. No. 17-1407-CFC-SRF
)	(CONSOLIDATED)
v.)	
)	
AMGEN INC.,)	
)	
<i>Defendant.</i>)	
_____)	

WORD COUNT CERTIFICATION

The undersigned counsel hereby certifies that the foregoing Reply Brief In Support of Genentech’s Motion to Dismiss and/or Strike Amgen’s Fifteenth Counterclaim and Fifteenth Affirmative Defense filed by Plaintiffs contains 2,106 words, in 14-point Times New Roman font, which were counted by Alexandra M. Joyce, Esq. using the word count feature in Microsoft Word.

DATED: April 29, 2020

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